New insights into an enigmatic tumour suppressor

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Dynamic regulation of the microtubule apparatus is central to cell division, cell migration, polarity and transport. New data demonstrating functional association of the von Hippel-Lindau (VHL) tumour suppressor with microtubules provide a new lead in unravelling VHL tumour suppressor mechanisms.

Von Hippel-Lindau disease is a hereditary cancer syndrome that predisposes people to tumours of the kidney (renal cell carcinoma (RCC)), retina and central nervous system (haemangioblastoma (HB)), the adrenal gland (phaeochromocytoma) and various cystic lesions mainly affecting the kidney and pancreas (for review, see ref. 1). Despite identification of the *VHL* gene on chromosome 3p25 almost ten years ago, the tumour suppressor mechanism remains poorly understood. On page 64 of this issue, Hergovich *et al.* now provide data indicating a new facet to VHL function[2]. They demonstrate association of the VHL gene product with the microtubule apparatus and suggest that mutations interfering with a microtubule-stabilizing function of VHL may contribute to the complex genotype–phenotype relationship that is observed in VHL disease.

VHL disease is one of a number of dominantly inherited cancer syndromes (other examples include familial retinoblastoma, Li-Fraumeni syndrome and familial adenomatous polyposis affecting the *Rb*, *TP53,* and *APC* genes, respectively) in which molecular analysis has confirmed the predictions of Knudson's classic 'two-hit' hypothesis. In such syndromes, affected families manifest germline transmission of a single predisposing mutant allele, with somatic inactivation of the second allele being required for cancer development. In general, functional analyses of the mutated tumour suppressor genes in such diseases have also conformed well to the predictions of classic cancer genetics. The relevant gene products have usually been shown to have clear roles in the control of cell proliferation or in genetic repair processes, and thus have been classified as gatekeepers or caretakers, respectively. Unusually, however, the VHL tumour suppressor has not yet been clearly accommodated within this model. The large number of benign multicellular lesions that are found in affected organs has suggested a gatekeeper mechanism. Nevertheless, indices of proliferation in such lesions are relatively low[3]. Moreover, the findings from direct assays of later aspects of VHL tumour suppressor function, in which VHL is re-introduced into fully transformed cells, are also unusual[4]. Re-expression of the wild-type *VHL* in *VHL*-defective RCC lines had no effect on monolayer growth but reduced growth of such cells as tumours in nude mice. This has indicated that the tumour suppressor action is in some way dependent on conditions manifested during solid tumour growth, but not during standard monolayer cell culture, fuelling interest in understanding abnormalities of cell–matrix and cell–cell interactions in these cells[5–7].

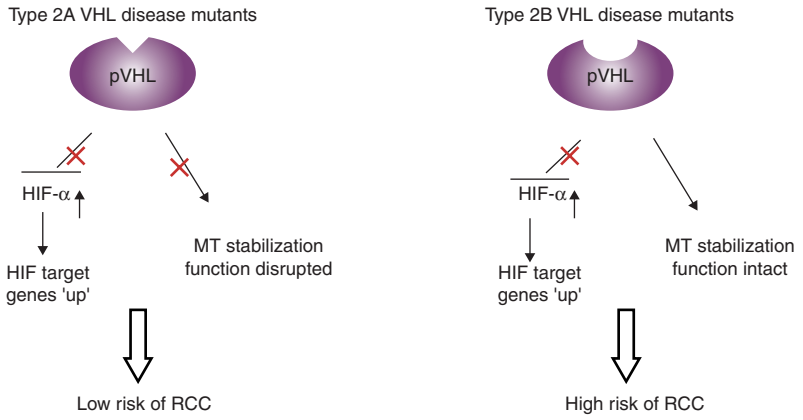

Figure 1 Model for different VHL functions and the risk of RCC disease sub-type.

The complex nature of VHL function is further suggested by the fact that clinical observations of VHL disease demonstrate a complex genotype–phenotype relationship. Families have been classified as type 1 or type 2, according to whether the risk of phaeochromocytoma is low or high[1] (Table 1). Definition of the mutational spectrum has indicated a clear bias against inactivating mutations in type-2 disease, suggesting either that the development of phaeochromocytoma is incompatible with complete loss of VHL function, or that some gain of function is acquired by VHL mutations that promote this type of tumour. Further complexity is added by striking differences in the risk of RCC and HB among families bearing type-2 mutations (Table 1). Taken together, these findings have suggested the operation of distinct, possibly tissue-specific, tumour suppressor mechanisms.

To what extent can these findings be explained by current knowledge of the molecular function of pVHL? To date, VHL is best understood as a ubiquitin ligase in the oxygen-dependent proteolytic degradation of the hypoxia inducible factor (HIF) transcriptional complex[8]. Although a critical physiological role for VHL in hypoxic gene regulation is established, the extent to which dysregulation of the HIF pathway accounts for cancer predisposition in VHL disease remains uncertain. Support for a tumour-promoting effect has been obtained (at least for one HIF isoform, HIF-2α) from rescue of the tumour suppressor effects of VHL by a mutant HIF-2α that escapes VHL-mediated proteolysis[9]. Nevertheless, direct mutational activation of HIF has not, so far, been described in relevant tumour types. This has suggested that oncogenic selective pressures might in fact be operating on different, as yet unknown, VHL-associated pathways.

In attempts to define such pathways, several studies have compared gene expression profiles and cellular phenotypes in *VHL*-defective RCC cells and transfectants re-expressing wild-type *VHL*. Such studies have implicated VHL in a variety of processes of potential relevance to its tumour suppressor

activity. These include cell cycle regulation by exogenous stimuli, extracellular matrix formation, cell–cell interactions, cell invasion, branching morphogenesis and epithelial organization[5–7,10,11]. A key question is whether these properties are connected with each other or with the hypoxia response, or whether they represent distinct actions of VHL that might reveal distinct tumour suppressor pathways.

How does the new work by Hergovich *et al.* fit with these findings? Using a combination of approaches, they revealed that cytoplasmic VHL is largely associated with the microtubule apparatus. A complex twist to this tale is that this association was specific to one VHL isoform only. Human pVHL exists as two isoforms: a protein with a relative molecular mass (M_r) of approximately 30,000 (30K; $pVHL_{30}$) that corresponds to the predicted 213-amino-acid product of the entire open reading frame and a second 19K isoform ($pVHL_{19}$) produced by translational initiation at an internal methionine at codon 54. The $pVHL_{19}$ isoform is competent for ubiquitin ligase activity[1], and, as all tumour-associated mutations affect $pVHL_{19}$ coding sequences, it has been assumed that this function is critical for tumour suppressor function.

Using two-colour confocal immunostaining, Hergovich *et al.* show differential localization of $pVHL_{19}$ and $pVHL_{30}$ isoforms, demonstrating that although $pVHL_{19}$ is predominantly localized to the nucleus, the longer $pVHL_{30}$ isoform is largely localized to the microtubule apparatus. In addition, the localization of $pVHL_{30}$ is markedly perturbed by drugs that alter microtubule polymerization. The authors demonstrate a physicial association of VHL with microtubule proteins by cosedimentation with taxol-stabilized microtubule assemblies and by co-immunoprecipitation of α-tubulin with VHL. Moreover, using *in vivo* assays of microtubule stability, they show that VHL protects against nocodazole-induced microtubule dissassembly[2]. Interestingly, from mutational analysis, they found that this function is independent of E3 complex formation, suggesting that microtubule binding and stabilization may be an independent function of VHL.

To address the potential relationship to tumour suppressor function, they asked how *VHL* mutations associated with different patterns of tumour development affect microtubule stability. This approach has previously been used to analyse links between dysregulation of HIF and VHL disease subtypes and has supported a role of HIF activation in HB and RCC, but not phaeochromocytoma (see Table 1)[12,13]. In the current study, Hergovich *et al.* found that although many missense mutations retained the ability to stabilize microtubules, at least under assay conditions explored, this property was abrogated by two specific mutations, Y98H and Y112H. These mutations are associated with type-2A disease (a high risk of phaeochromocytoma and a low risk of RCC, see Fig. 1), a genotype–phenotype pattern that is quite distinct from the effects of VHL mutations on HIF. One possibility is that defective microtubule stabilization might contribute to the development of phaeochromocytoma. However, if this is the case, it is difficult to understand why alternative substitutions at Tyr 98 and Tyr 112 that are associated with type 2B disease (a high risk of phaeochromocytoma and RCC, see Fig. 1) were found to behave normally in these assays and why mutations that affect $pVHL_{19}$ seem to be necessary for tumour-promoting effects. This suggests a different explanation — that the mutational effect somehow mitigates against the RCC predisposition. How can this hypothesis be squared with the high risk of RCC associated with completely inactivating VHL mutations? The authors noted that although the Y98H and Y112H mutants are dysfunctional in microtubule stability assays, they continue to bind the microtubule apparatus, suggesting that the low risk of RCC may represent a gain-of-function effect of these mutations.

More work will be needed to prove such an effect and to define the mechanism. However, irrespective of whether this precise explanation holds up, the new finding that VHL associates with the microtubule apparatus is convincing and provides an important lead in understanding VHL function. Interestingly, another tumour suppressor (the adenomatous polyposis coli, *APC* gene product) is known to interact with the microtubule apparatus, and existing data on the APC–microtubule interaction could be of interest in directing new studies of VHL. Similarly to VHL, APC has a well-defined proteolytic function, regulating the Wnt signalling pathway through the ubiquitin-mediated degradation of β-catenin. However, accumulating evidence has indicated additional or connected roles for APC in microtubule functions. APC promotes microtubule polymerization *in vitro* and *in vivo* and preferentially localizes to the plus (growing) ends via associations with another micotubule-associated protein EB1 (ref. 14). Although the role of defective microtubule functions in APC tumour suppressor behaviour is unclear, localization studies have stimulated two lines of enquiry. In mitotic cells, APC–EB1 complexes localize to the spindle–kinetochore connection and it has been proposed that defective attachment results in a chromosomal instability phenotype that is observed in *APC*-defective cells[15]. In interphase cells, APC has been shown to cluster at the plus ends of microtubules near the plasma membrane. The association of these clusters with the formation of cellular extensions and alterations in the response to 'wound healing' of confluent cultures have suggested an involvement in the regulation of cell motility[16]. Given the existing focus on abnormal cell–matrix interactions and epithelial organization in *VHL*-defective cells, the new data demonstrating a VHL–microtubule association provides an interesting new lead in the pursuit of this enigmatic tumour suppressor function. □

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Table 1 Genotype–phenotype associations in VHL disease

	Type 1	Type 2		
		A	B	C
Tumours				
Phaeochromocytoma	–	+	+	+
RCC	+	–	+	–
HAB	+	+	+	–
Funtional assays				
HIF proteolysis	–	–	–	+
MT stabilization	?	–	+	+

1. Kaelin, W. G. *Nature Rev. Cancer* **2**, 673–682 (2002).
2. Hergovich, A. *et al. Nature Cell Biol.* **4**, 64–70 (2002).
3. Mandriota, S. J. *et al. Cancer Cell* (in the press).
4. Iliopoulos, O., Kibel, A., Gray, S. & Kaelin, W. G. Jr. *Nature Med.* **1**, 822–826 (1995).
5. Koochekpour, S. *et al. Mol. Cell. Biol.* **19**, 5902–5912 (1999).
6. Ohh, M. *et al. Mol. Cell* **1**, 959–968 (1998).
7. Davidowitz, E. J., Schoenfeld, A. R. & Burk, R. D. *Mol. Cell. Biol.* **21**, 865–874 (2001).
8. Maxwell, P. H. *et al. Nature* **399**, 271–275 (1999).
9. Kondo, K. *et al. Cancer Cell* **1**, 237–246 (2002).
10. Bindra, R. S. *et al. Cancer Res.* **62**, 3014–3019 (2002).
11. Esteban-Barragan, M. A. *et al. Cancer Res.* **62**, 2929–2936 (2002).
12. Clifford, S. C. *et al. Hum. Mol. Genet.* **10**, 1029–1038 (2001).
13. Hoffman, M. A. *et al. Hum. Mol. Genet.* **10**, 1019–1027 (2001).
14. Nakamura, M., Zhou, X. Z. & Lu, K. P. *Curr. Biol.* **11**, 1062–1067 (2001).
15. Pellman, D. *Science* **291**, 2555–2556 (2001).
16. Mimori-Kiyosue, Y., Shiina, N. & Tsukita, S. *J. Cell Biol.* **148**, 505–517 (2000).